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APPROXIMATIVE CALCULATION OF THE BUFFER BASE, THE TITRATION CURVE, AND CO_2-DISSOCIATION CURVE OF BRAIN TISSUE

T. Middendorf and H.H. Loeschcke

Translation of "Approximative Berechnung der Pufferbase, einer Titrationsgeraden für Proteine und der CO_2-Dissoziationskurve des Gehirngewebes," Pflügers Archiv, Vol. 349, No. 1, 1974, pp. 1-8

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16. Abstract An analysis of the acid-base balance and the CO_2-binding capacity of the brain is presented. It is based on a linear titration curve for the cerebral proteins, the mass action laws for the first dissociation of carbonic acid and the second dissociation of phosphoric acid, the condition of electrical neutrality and finally the experimental buffer line ($pH = a \log P_{BCO_2} + b$) based on the data of Kjällquist et al. (1969), the total phosphate ion and protein concentration of McIlwain and Bachelard (1971). The following values for the slope of the protein titration curve ($d[P^-]/dpH$), an average isoelectric point of the proteins involved and the buffer base of the whole brain were obtained: 37.18 meq/ /kg $H_2O \cdot pH$; 5.718; 77 meq/kg H_2O. The CO_2 dissociation curve derived from these data approximates the experimental data of Kjällquist et al.

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APPROXIMATIVE CALCULATION OF THE BUFFER BASE, THE TITRATION CURVE, AND CO_2-DISSOCIATION CURVE OF BRAIN TISSUE

Traugott Middendorf and Hans H. Loeschcke,
Institute for Physiology, Ruhr University, Bochum, Germany

One of the first articles in which in vivo CO_2 concentrations /1* in brain tissue were determined experimentally during acute respiratory acidosis is that by Thompson and Brown (1960). Systemization is provided in the article by Pontén (1964), whose experimentally obtained buffer line is described by the equation $pH = -0.79 \log P_{BCO_2} + 8.$ [illegible] Subscript B stands for brain tissue. In 1966, Pontén published an additional article on cerebral CO_2 binding with a confirmation of his earlier finding that a quasi-steady state in CO_2 binding is reached in approximately 30 min. On the other hand, he obtains a buffer line on the basis of a modified freezing technique which differs basically from that obtained earlier in its slope ($pH = -0.674 \log P_{BCO_2} + 8.303$). In contrast to Pontén's work, Weyne et al. (1968) and Kjällquist et al. (1969) find equivalent buffer lines for the entire brain in the normocapnic and hypercapnic ranges, while $-d \log P_{BCO_2}/dpH$ increases /2 rapidly under hypocapnic conditions, due to a change in the buffer base. It is shown to be probable that this occurs as the result of lactate production. Buffer capacity and short-term chemical shifts in milieu, rather than long-term ionic exchange processes between intracellular and extracellular phases and certainly not renal compensation mechanisms (Brown, 1971), are responsible for the CO_2 dissociation curves determined experimentally under acute conditions by the above authors.

In contrast to Pontén (1964, 1966) and Weyne et al. (1968), who plot a buffer line for total cerebral fluid, Kjällquist et al.

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* Numbers in the margin indicate pagination in the foreign text.

(1969) describe CO_2 binding in extracellular and intracellular fluids of the brain separately in rats. Since according to Kjällquist et al., Pontén's and Weyne's values for buffer capacity are too small as the result of the anesthesia used, Kjällquist's findings will be used as the basis here.

A summary of the relationships between protein content, buffer base and CO_2 binding in the brain is provided by Siesjö and Pontén (1966). In this article, buffering by protein is treated like buffering by a univalent acid-salt system A^-/HA. Proteins contain many and varying groups, however, which behave as proton acceptors; this is manifested in the fact that the pH-meter titration curve can be approximated better with a straight line than with the S curve characteristic of an individual dissociating group. For this reason, it appeared more realistic to us to base the formulation of buffering by proteins on a linear titration curve. In addition, a phosphate buffer is covered in this article, since according to McIlwain and Bachelard (1971), appreciable phosphate concentrations exist in the brain.

Theory and Results

The water content of the rat brain is 78.4%. This includes about 3% blood water and 12% extracellular fluid (Pontén, 1966, and Kjällquist et al., 1969). We then find bicarbonate concentration in the brain $[HCO_3^-]_B$ to be

$$[HCO_3^-]_B = \frac{0.634\,[HCO_3^-]_{ICF} + 0.120\,[HCO_3^-]_{ECF}}{0.754} \qquad (1)$$

in mmol/kg H_2O (63.4%, 12% and 75.4% water content in intracellular and extracellular spaces and in the two combined, without blood). The associated cerebral mixed pH_B for $P_{B_{CO_2}}$ and $[HCO_3^-]_B$ can be determined with the aid of the Henderson-Hasselbach

equation. We obtain the following table of values from the data of Kjällquist et al. (1969):

$P_{B_{CO_2}}$	$\log P_{B_{CO_2}}$	$[HCO_3^-]_B$	pH_B
20	1,301	8,587	7,256
30	1,477	11,905	7,222
40	1,602	14,140	7,171
50	1,699	16,062	7,130
60	1,778	17,658	7,092
70	1,845	19,021	7,057
80	1,903	20,353	7,029
90	1,954	21,537	7,002

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[Note: Commas in numerals are equivalent to decimal points.]

The last six values in this table can be obtained approximately with the Astrup or buffer line.

$$pH_B = -0.483 \log P_{B_{CO_2}} + 7.948 \qquad (2)$$

If we apply the Henderson-Hasselbach equation to (2), we obtain the expression

$$[HCO_3^-]_B = 10^{-1.070\ pH_B + 8.832} \qquad (2a)$$

for $[HCO_3^-]_B$ as a function of pH_B; this can be approximated with a line for pH_E [7.0, 7.18]:

$$[HCO_3^-]_B = -43{,}29\,(pH - 7{,}50) \qquad (2b)$$

The buffer or Astrup line is determined physicochemically by the protein-buffer system P^-/P, the phosphate-buffer system

$HPO_4^=/H_2PO_4^-$ and the carbon dioxide buffer system HCO_3^-/CO_2.

$$[P^-] = A * P * (pH - pH_0) \quad \text{Titration line} \quad (3)$$

$$pH = pK_{Ph_2} + \log [HPO_4^=] - \log [H_2PO_4^-] \quad \text{Law of mass action for phosphate} \quad (4)$$

$$pH = pK' + \log [HCO_3^-] - \log [CO_2] \quad \text{Henderson-Hasselbach equation,} \quad (5)$$

where P is total protein concentration in g/100 ml, A is a slope factor in $\frac{meq}{kg\ H_2O} \cdot \frac{100\ ml}{g} \cdot \frac{1}{pH}$, pK_{Ph2} is the second dissociation constant of phosphoric acid, pK' is the hydration and dissociation constant of carbon dioxide / bicarbonate, and pH_o is the pH at which the sum of positive and negative charges on protein is zero. According to Netter (1959), $pK_{Ph_2} = 6.81$ and, according to Kjällquist et al. (1969), pK' = 6.12. The slope and pH_o of the protein titration line are unknown.

In the physiologically meaningful region, we can take /4
$d[HCO_3^-]/dpH = -43.29$ from equation (2b). This value is further affected by the protein and phosphate buffer systems. On the basis of electron neutrality and the stoichiometry of CO_2 binding, we have

$$d[HCO_3^-] = -(d[P^-] + d[HPO_4^=])$$
$$\frac{d[HCO_3^-]}{dpH} = -\left(\frac{d[P^-]}{dpH} + \frac{d[HPO_4^=]}{dpH}\right) \quad (6)$$

The slope of the protein titration curve being sought now appears in (6).

Using equation (4) (Loeschcke, 1972) and the condition of constancy of the sum of phosphate $[H_2PO_4^-] + [HPO_4^=] = C$ mmol/kg H_2O we can now determine $d[HPO_4^=]/dpH$. We obtain

$$\left| \frac{d\,[HPO_4^=]}{dpH} = \frac{[HPO_4^=]\,(C - [HPO_4^=])}{0.4343 \cdot C} \right| \qquad (7)$$

According to McIlwain and Bachelard (1971), C* = 16 meq/ℓ brain or C* = 21.22 meq/kg H_2O. If we assume that C* was determined at P_{BCO_2} = 46 torr or at a mixed pH value of 7.145, we obtain C = 12.60 mmol/kg H_2O.

From (7) we can then write equation (6) in the form

$$\left| \frac{d\,[P^-]}{dpH} = -\frac{d\,[HCO_3^-]}{dpH} - \frac{[HPO_4^=]\,(C - [HPO_4^=])}{0.4343 \cdot C} \right| \qquad (8)$$

With a standard bicarbonate value of $[HCO_3^-]_B$ = 14.316 mmol/kg H_2O and a pH of pH_B = 7.174, we obtain an $[HPO_4^=]$ value of 8.796 mmol/kg H_2O, i.e.

$$\left| \frac{d\,[P^-]}{dpH} = 43.29 - 6.11 = 37.18 \frac{meq}{kg\ H_2O} \cdot \frac{1}{pH} \right|$$

(In this formula, meq is correct if only $HPO_4^=$ and not $H_2PO_4^-$ acts as the buffer. Then meq = mmol; referred to protein, however, presentation in meq is to be preferred.) Thus the slopes of titration lines for cerebral proteins are known to a first approximation. In order to now determine pH_o, it is necessary to know at least one $[P^-]$ value and the associated pH. According to McIlwain and Bachelard (1971), overall cation concentration in the human brain is 166 meq/ℓ and anion concentration without proteins and lipids is 87 meq/ℓ. The difference between cation and anion concentrations is divided equally, according to McIlwain and Bachelard (1971), between lipids and proteins. If it is correct that the lipids do not come under consideration for buffering, it is only necessary to consider protein. We then find dissociated protein /5
concentration to be 40 meq/ℓ or 53.05 meq/H_2O at a cerebral mixed pH of 7.145. From these data we calculate pH_o = 5.718.

If we now assume, in accordance with McIlwain and Bachelard (1971), protein concentration in the brain to be 8 g/100 ml, we then obtain

$$A = 4{,}648 \frac{\text{meq}}{\text{kg } H_2O} \cdot \frac{100 \text{ ml}}{\text{g}} \cdot \frac{1}{\text{pH}} \cdot$$

In order to now obtain the CO_2 dissociation curve formally, we begin with the electron neutrality condition for cerebral fluid (Siesjö and Pontén, 1966). If $[Cat^+]$ refers to cations other than $[H^+]$ and $[An^-]$ (including $H_2PO_4^-$) refers to anions, other than the buffer base, consisting of $[P^-]$, $[HPO_4^-]$ and $[HCO_3^-]$, then

$$[Cat^+] + [H^+] = [An^-] + [HCO_3^-] + [HPO_4^-] + [P^-].$$

The milliequivalent values are to be substituted into this equation, i.e. since $[HPO_4^=]$ has previously been expressed in mmol/kg H_2O, it must be multiplied by a factor of 2 here.

Since $[Cat^+] - [An^-] - \frac{1}{2}[HPO_4^-] = [PB]$ it follows, if we take equations (3), (4) and (5) into consideration, that

$$[PB] = -K' \frac{S_{CO_2} \cdot P_{CO_2}}{[HCO_3^-]} + [HCO_3^-] + \frac{K_{Ph_2} \cdot C}{[H^+] + K_{Ph_2}} + A \cdot P \cdot (pH - pH_0)$$

or

$$[PB] = -K' \frac{S_{CO_2} \cdot P_{CO_2}}{[HCO_3^-]} + [HCO_3^-] + \frac{K_{Ph_2} \cdot C \cdot [HCO_3^-]}{K' \cdot S_{CO_2} \cdot P_{CO_2} + K_{Ph_2} \cdot [HCO_3^-]} + A \cdot P \left(pK' + \log \frac{[HCO_3^-]}{S_{CO_2} \cdot P_{CO_2}} - pH_0\right) \qquad (9)$$

If we apply (2) and (2a) or (2b) to (9), we find the cerebral buffer base to be 77 meq/kg H_2O. If we now solve (9) with respect to $\log P_{CO_2}$, assuming $[H^+]$ to be negligibly small, we obtain the following equation for CO_2 dissociation for the cerebral fluid:

$$\log P_{B_{CO_2}} = \frac{1}{A \cdot P}\left([HCO_3^-]_B + \frac{K_{Ph_2} \cdot C \cdot [HCO_3^-]_B}{K' \cdot S_{CO_2} \cdot P_{B_{CO_2}} + K_{Ph_1} \cdot [HCO_3^-]_B} - [PB]_B\right) + pK' + \log [HCO_3^-]_B - pH_0 - \log S_{B_{CO_2}}$$

or

$$\log P_{B_{CO_2}} = \frac{1}{37.18}\left([HCO_3^-]_B + \frac{10^{-6.81} \cdot 12.60 \cdot [HCO_3^-]_B}{10^{-6.12} \cdot 0.0314 \cdot P_{B_{CO_2}} + 10^{-6.81} \cdot [HCO_3^-]_B} - 77\right) + 6{,}12 + \log [HCO_3^-]_B - 5{,}718 - \log 0{,}0314 .$$

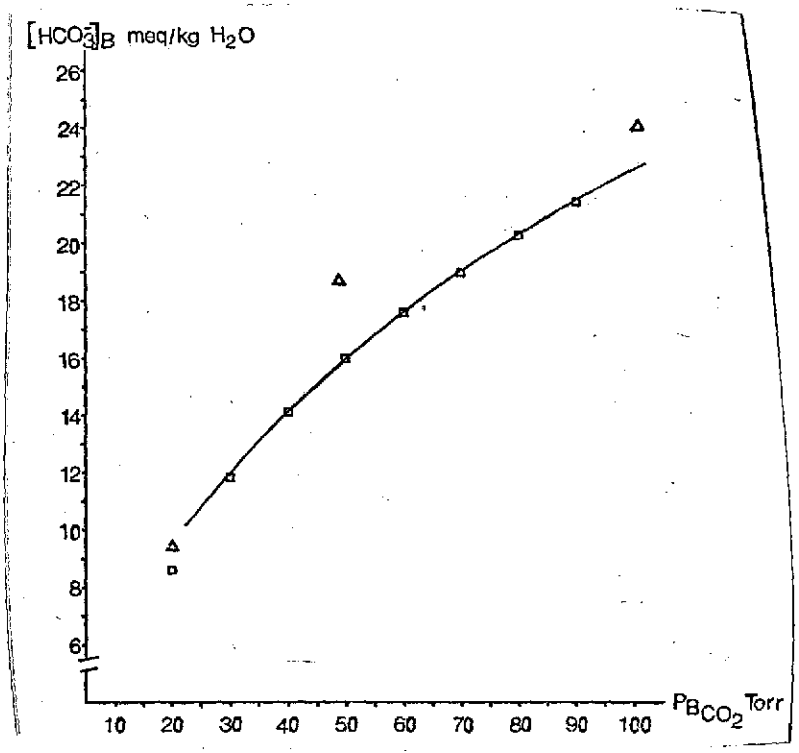

Fig. 1. Experimental (Weyne et al, 1968; Kjällquist et al, 1969) and theoretical relationships between bicarbonate concentration and CO_2 pressure in brain tissue. The deviation of the experimental data for hypocapnia and in those obtained by Weyne et al. (1968) from the calculated CO_2 dissociation curves is due to an altered Astrup line. Δ Weyne et al. (1968); □ Kjällquist et al (1969); — theory.

Fig. 1 shows a comparison between calculated and experimental values. It is found that the experimental data from Kjällquist et al. (1969) is approximated quite well by the calculated values for normocapnic and hypocapnic conditions. The relatively large deviation in the data for hypocapnia and in those from Weyne et al. (1968) is due to an altered buffer line. Although it was expected that the theoretical and experimental results would agree, this agreement is not based on a pure approximation to the empirical data, but on the applicability of the assumed physicochemical laws and the empirical data such as the buffer line from Kjällquist et al. (1969) and total phosphate

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ion and dissociated protein concentrations from McIlwain and Bachelard (1971).

Discussion /7

Siesjö and Pontén (1966) likewise attempted, as mentioned above, a theoretical description of CO_2 binding in brain tissue. Since they did not base their considerations on a titration line for the protein buffer system and a phosphate buffer, but merely used the simple law of mass action for protein, a comparison of numerical values is hardly possible. On the basis of his buffer line, Pontén (1966) arrives at a value of 36 meq/kg H_2O for the buffer base, a value which is probably too small, if we consider that Pontén's (1966) buffer capacity corresponds approximately to that of the blood, $d \log P_{CO_2}/dpH = -1.5$ (Siesjö and Pontén, 1966) and, under these given conditions, the blood has a buffer base of approximately 50 meq/ℓ. According to Altman and Dittmer (1971), the buffer base of erythrocytes is 57 meq/ℓ erythrocytes or 81 meq/kg H_2O. A comparison with the buffer base of 77 meq/kg H_2O calculated from the data from Kjällquist et al. (1969) indicates that buffering in the brain is probably just as good as in red blood cells, although protein concentration in the brain, 8 g/100 ml, is somewhat lower than in erythrocytes, with an Hb concentration of 33 g/100 ml.

It should be noted, on the other hand, that the description given here of the buffer base consisting of HCO_3^-, HPO_4^- [sic], P^- and the pH_o value of 5.718 are very approximate in character, since, for example, the composition of the buffer, the correct amount of dissociated protein for a pH value, the shape of the titration curve for brain proteins and the like are known only approximately. It should be mentioned, moreover, that the theoreticaly derivation of the CO_2 dissociation curve is valid only for acute cases and not for more prolonged ones, for which

altered CO_2 dissociation curves are obtained, according to Kazemi et al. (1967) and Weyne et al. (1968).

However, the proposed method appears to be suitable and adequate for calculating the parameters of acid-base equilibrium in the brain under the condition of acute changes in CO_2 pressure.

REFERENCES

Altman, P.L., and Dittmer, D. S., Blood and Other Body Fluids, Federation of American Societies for Experimental Biology, Bethesda, Maryland, 1971, p. 189.

Brown, E.B., Jr., "Whole body buffer capacity," in Alfred Benzon Symposium III: Ion Homeostasis of the Brain, Copenhagen: Munksgaards, 1971, pp. 317-333.

Kazemi, H., Shannon, D.C. and Carvallo-Gil, E., "Brain CO_2 buffering capacity in respiratory acidosis and alkalosis," J. Appl. Physiol. 22, 241-246 (1967).

Kjällquist, A., Nardini, M., and Siesjö, B. K., "The regulation of extra- and intracellular acid-base parameters in the rat brain during hyper- and hypocapnia," Acta Physiol. Scand. 76, 485-494 (1969).

Loeschcke, H.H., "Acid-base status in the liquor cerebrospinalis and its regulation by lung ventilation," Klin. Wochenschr. 50, 581-593 (1972). /8

McIlwain, H. and Bachelard, H. S., Biochemistry and the Central Nervous System, Edinburgh-London: Chruchill Livingstone, 1971.

Netter, H., Theoretische Biochemie [Theoretical Biochemistry], Berlin-Göttingen-Heidelberg, Springer, 1959.

Pontén, U., "The CO_2-binding capacity of rat brain tissue in vivo," Experientia (Basel) 20, 287-289 (1964).

Pontén, U., "Acid-base changes in rat brain tissue during acute respiratory acidosis and baseosis," Acta Physiol. Scand. 68, 152-163 (1966).

Seisjö, B.K. and Pontén, U., "The buffer capacity of brain tissue and of equivalent systems," N.Y. Acad. Sci. 133, 180-189 (1966).

Thompson, A.M. and Brown, E.B., Jr., "Tissue carbon dioxide concentrations in rats during acute respiratory acidosis," J. Appl. Physiol. 15, 49-52 (1960).

Weyne, J., Demeester, G. and Leusen, I., "Bicarbonate and chloride shifts in rat brain during acute and prolonged respiratory acid-base changes," Arch. Int. Physiol. 75, 415-433 (1968).